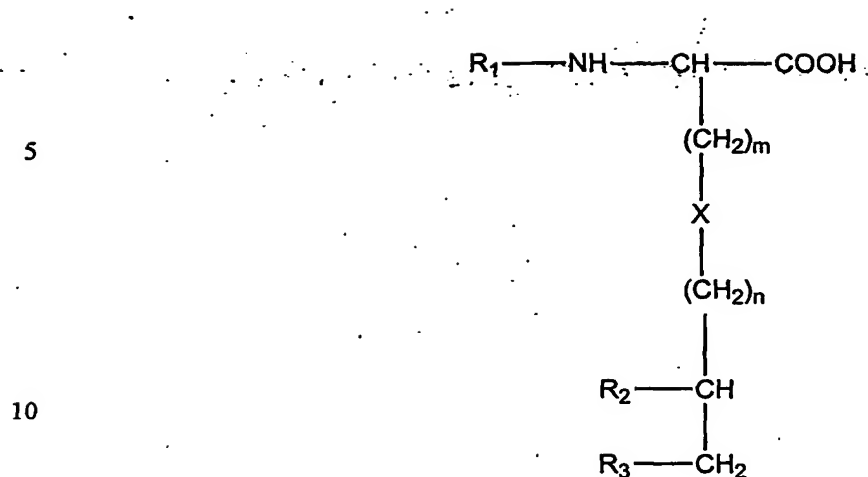


WE CLAIM:

1. A lipopeptide comprising a polypeptide conjugated to one or more lipid moieties wherein:
 - 5 (i) said polypeptide comprises an amino acid sequence that comprises:
 - (a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a cytotoxic T cell (CTL) epitope, wherein said amino acid sequences are different; and
 - (b) one or more internal lysine residues or internal lysine analog residues
10 for covalent attachment of each of said lipid moieties via the epsilon-amino group or terminal side-chain group of said lysine or lysine analog; and
 - (ii) each of said one or more lipid moieties is covalently attached to an epsilon-amino group of said one or more internal lysine residues or to a terminal side-chain group of said one or more internal lysine analog residues.
- 15 2. The lipopeptide of claim 1 wherein the lipid is attached to the epsilon-amino group of a lysine residue.
3. The lipopeptide of claim 1 or 2 wherein the internal lysine residue to which
20 a lipid moiety is attached is positioned between the Th epitope and the CTL epitope.
4. The lipopeptide of claim 1 or 2 wherein the internal lysine residue to which a lipid moiety is attached is positioned within the Th epitope.
- 25 5. The lipopeptide according to any one of claims 1 to 4 wherein the lipid moiety has a structure of General Formula (VII):

Formula (VII)



wherein:

- (i) X is selected from the group consisting of sulfur, oxygen, disulfide (-S-S-), and methylene (-CH₂-), and amino (-NH-);
- (ii) m is an integer being 1 or 2;
- (iii) n is an integer from 0 to 5;
- (iv) R₁ is selected from the group consisting of hydrogen, carbonyl (-CO-), and R'-CO- wherein R' is selected from the group consisting of alkyl having 7 to 25 carbon atoms, alkenyl having 7 to 25 carbon atoms, and alkynyl having 7 to 25 carbon atoms, wherein said alkyl, alkenyl or alkynyl group is optionally substituted by a hydroxyl, amino, oxo, acyl, or cycloalkyl group;
- (v) R₂ is selected from the group consisting of R'-CO-O-, R'-O-, R'-O-CO-, R'-NH-CO-, and R'-CO-NH-, wherein R' is selected from the group consisting of alkyl having 7 to 25 carbon atoms, alkenyl having 7 to 25 carbon atoms, and alkynyl having 7 to 25 carbon atoms, wherein said alkyl, alkenyl or alkynyl group is optionally substituted by a hydroxyl, amino, oxo, acyl, or cycloalkyl group; and
- (vi) R₃ is selected from the group consisting of R'-CO-O-, R'-O-, R'-O-CO-, R'-NH-CO-, and R'-CO-NH-, wherein R' is selected from the group consisting of alkyl having 7 to 25 carbon atoms, alkenyl having 7 to 25 carbon atoms, and alkynyl having 7 to 25 carbon atoms, wherein said alkyl, alkenyl or alkynyl group is optionally substituted by a hydroxyl, amino, oxo, acyl, or cycloalkyl group

and wherein each of R₁, R₂ and R₃ are the same or different.

6. The lipopeptide of claim 5 wherein X is sulfur; m and n are both 1; R₁ is selected from the group consisting of hydrogen, and R'-CO-, wherein R' is an alkyl group having 7 to 25 carbon atoms; and R₂ and R₃ are selected from the group
 5 consisting of R'-CO-O-, R'-O-, R'-O-CO-, R'-NH-CO-, and R'-CO-NH-, wherein R' is an alkyl group having 7 to 25 carbon atoms.

7. The lipopeptide of claim 6 wherein R' is selected from the group consisting of: palmitoyl, myristoyl, stearoyl, lauroyl, octanoyl, decanoyl, and cholesterol.

10

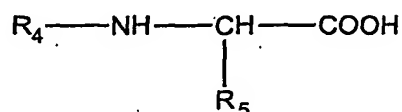
8. The lipopeptide according to any one of claims 5 to 7 wherein the lipid is contained within a lipoamino acid moiety selected from the group consisting of: Pam₁Cys, Pam₂Cys, Pam₃Cys, Chol₂Lys, Ste₂Cys, Lau₂Cys, and Oct₂Cys.

15 9. The lipopeptide according to claim 8 wherein the lipoamino acid moiety is Pam₂Cys.

10. The lipopeptide according to any one of claims 1 to 4 wherein the lipid moiety has the following General Formula (VIII):

20

Formula (VIII)



25 wherein:

- (i) R₄ is selected from the group consisting of: (i) an alpha-acyl-fatty acid residue consisting of between about 7 and about 25 carbon atoms; (ii) an alpha-alkyl-beta-hydroxy-fatty acid residue; (iii) a beta-hydroxy ester of an alpha-alkyl-beta-hydroxy-fatty acid residue; and (iv) a lipoamino acid
 30 residue; and
- (ii) R₅ is hydrogen or the side chain of an amino acid residue.

11. The lipopeptide according to any one of claims 1 to 10 wherein the lipid moiety is separated from the peptide moiety by a spacer.

12. The lipopeptide of claim 11 wherein the spacer comprises arginine, serine or 6-aminohexanoic acid.
- 5 13. The lipopeptide of claim 11 or 12 wherein the spacer consists of a serine homodimer.
14. The lipopeptide according to any one of claims 1 to 13 wherein the internal lysine or internal lysine analog is nested within a synthetic amino acid sequence
10 having low immunogenicity.
15. The lipopeptide according to any one of claims 1 to 14 wherein the T-helper epitope is a T-helper epitope of influenza virus haemagglutinin or a T-helper epitope of canine distemper virus F (CDV-F) protein.
- 15 16. The lipopeptide of claim 15 wherein the T-helper epitope of influenza virus haemagglutinin comprises the amino acid sequence set forth in SEQ ID NO: 1.
17. The lipopeptide of claim 15 wherein the T-helper epitope of CDV-F protein
20 comprises the amino acid sequence set forth in SEQ ID NO: 20.
18. The lipopeptide according to any one of claims 1 to 17 wherein the CTL epitope is from an immunogenic protein, lipoprotein, or glycoprotein of a virus.
- 25 19. The lipopeptide according to claim 18 wherein the virus is influenza virus.
20. The lipopeptide of claim 19 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 2.
- 30 21. The lipopeptide according to claim 18 wherein the virus is hepatitis C virus.
22. The lipopeptide of claim 21 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 176.

23. The lipopeptide according to any one of claims 1 to 17 wherein the CTL epitope is from an immunogenic protein, lipoprotein, or glycoprotein of a prokaryotic organism.

5 24. The lipopeptide according to claim 23 wherein the CTL epitope is from *Listeria monocytogenes*.

25. The lipopeptide of claim 24 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 172.

10

26. The lipopeptide according to any one of claims 1 to 17 wherein the CTL epitope is from an immunogenic protein, lipoprotein, or glycoprotein of a eukaryotic organism.

15 27. The lipopeptide according claim 26 wherein the eukaryotic organism is a parasite.

28. The lipopeptide according to claim 26 wherein the eukaryotic organism is a mammal.

20

29. The lipopeptide according to claim 28 wherein the CTL epitope is from an ovalbumin protein of a mammal or a tumor cell.

25 30. The lipopeptide according to claim 29 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 173.

30 31. The lipopeptide according to any one of claims 1 to 30 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 174, SEQ ID NO: 175 and SEQ ID NO: 177.

32. The lipopeptide according to any one of claims 1 to 31 capable of upregulating the surface expression of MHC class II molecules on immature dendritic cells (DC).

33. The lipopeptide of claim 32 wherein the DC are D1 cells.

34. A lipopeptide comprising a polypeptide conjugated to one or more lipid
5 moieties wherein:

(i) said polypeptide comprises an amino acid sequence that comprises:

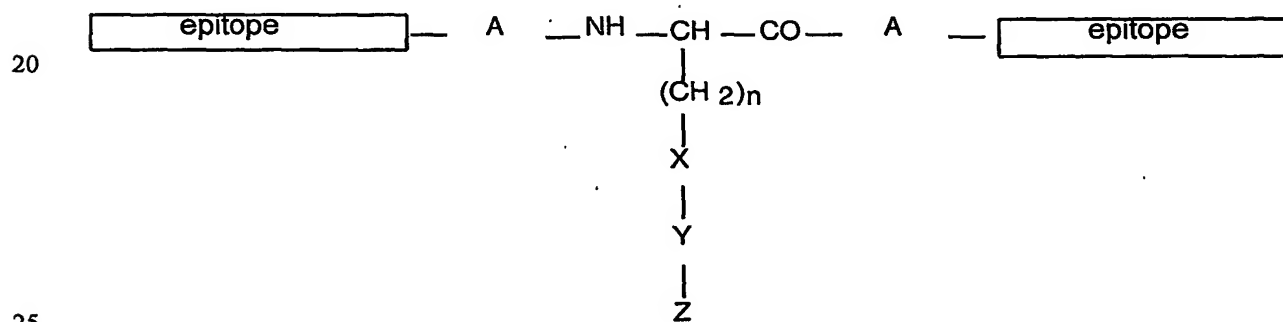
(a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a CTL epitope, wherein said amino acid sequences are different; and

10 (b) one or more internal lysine or lysine analogue residues for covalent attachment of each of said lipid moieties via the epsilon-amino group of said one or more lysine or lysine analogue residues;

(ii) each of said one or more lipid moieties is covalently attached to an epsilon-amino group of said one or more internal lysine residues; and

15 (iii) said lipopeptide has the general Formula (VI):

Formula (VI):



wherein:

epitope is a T-helper epitope or CTL epitope;

A is either present or absent and consists of an amino acid spacer of about 1 to about 6 amino acids in length;

30 n is an integer having a value of 1, 2, 3, or 4;

X is a terminal side-chain group selected from the group consisting of NH, O and S;

Y is either present or absent and consists of an amino acid spacer of about 1 to about 6 amino acids in length; and

Z is a lipid moiety.

35. The lipopeptide of claim 34 wherein A is absent.

5 36. The lipopeptide of claim 34 or 35 wherein Y is present and consists of a serine homodimer.

37. The lipopeptide according to any one of claims 34 to 36 wherein Z is selected from the group consisting of: Pam₁Cys, Pam₂Cys, Pam₃Cys, Chol₂Lys,
10 Ste₂Cys, Lau₂Cys, and Oct₂Cys.

38. The lipopeptide according to any one of claims 34 to 37 capable of upregulating the surface expression of MHC class II molecules on immature dendritic cells (DC).
15

39. The lipopeptide of claim 38 wherein the DC are D1 cells.

40. A method of producing a lipopeptide comprising:

(i) producing a polypeptide comprising an amino acid sequence that
20 comprises:

(a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a CTL epitope, wherein said amino acid sequences are different; and

(b) one or more internal lysine residues or internal lysine analog
25 residues; and

(ii) covalently attaching each of said one or more lipid moieties directly or indirectly to an epsilon-amino group of said one or more internal lysine residues or to the terminal side-chain group of said one or more internal lysine analog residues so as to produce a lipopeptide having the lipid
30 moiety attached to the epsilon amino group of said internal lysine residue or having the lipid moiety attached to the terminal side-chain group of said internal lysine analog residue.

41. The method of claim 40 wherein the polypeptide is synthesized by a chemical synthesis means.
42. The method of claim 40 or 41 further comprising producing the lipid moiety.
- 5 43. The method of claim 42 comprising synthesizing the lipid moiety as a lipoamino acid.
44. The method according to claim 43 further comprising adding a spacer to the
- 10 amino acid moiety of the lipoamino acid.
45. The method according to claim 44 wherein the spacer comprises an arginine homodimer or serine homodimer or 6-aminohexanoic acid .
- 15 46. The method of claim 44 or 45 comprising adding the spacer to the lipoamino acid via the terminal carboxy group in a process that comprises performing a condensation, addition, substitution, or oxidation reaction.
47. The method according to any one of claims 44 to 46 wherein the spacer
- 20 comprises a terminal protected amino acid residue to facilitate conjugation of the lipoamino acid to a polypeptide.
48. The method of claim 47 further comprising de-protecting the terminal protected amino acid of the spacer and conjugating the lipoamino acid to a
- 25 polypeptide.
49. The method of claim 43 comprising adding a spacer to a non-modified epsilon amino group of the polypeptide in a process comprising performing a nucleophilic substitution reaction.
- 30 50. The method of claim 49 wherein the polypeptide has an amino acid sequence comprising a single internal lysine or lysine analog residue and a blocked N-terminus.

51. The method according to claim 49 or 50 wherein the spacer comprises an arginine homodimer or serine homodimer or 6-aminohexanoic acid .
52. A composition comprising the lipopeptide according to any one of claims 1
5 to 39 and a pharmaceutically acceptable excipient or diluent.
53. The composition of claim 52 further comprising a biologic response modifier (BRM).
- 10 54. A method of eliciting an immune response in a subject comprising administering the lipopeptide according to any one of claims 1 to 39 or the composition according to claim 52 or claim 53 to said subject for a time and under conditions sufficient to elicit a cytotoxic T cell response against a CTL epitope in the lipopeptide.
- 15 55. The method according to claim 54 wherein the lipopeptide is administered intranasally to the subject.
56. The method according to claim 54 wherein the lipopeptide is administered
20 to the subject by injection.
57. A method of immunizing a subject against influenza virus comprising administering to said subject a lipopeptide comprising a polypeptide conjugated to one or more lipid moieties, wherein:
- 25 (i) said polypeptide comprises:
- (a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a CTL epitope of an influenza virus protein, and wherein said amino acid sequences are different;
 - (b) one or more internal lysine residues or internal lysine analog
30 residues for covalent attachment of each of said lipid moieties via an epsilon-amino group of said internal lysine or via a terminal side-chain group of said internal lysine analog; and
 - (c) each of said one or more lipid moieties is covalently attached directly or indirectly to an epsilon-amino group of said one or more

internal lysine residues or to a terminal side-chain group of said one or more internal lysine analog residues; and

- (ii) said lipopeptide is administered for a time and under conditions sufficient to elicit a CTL response to said CTL epitope.

5

58. The method of claim 57 wherein the lipopeptide is administered in combination with a pharmaceutically acceptable excipient or diluent.

59. The method of claim 57 or 58 wherein immunological memory is generated against the CTL epitope.

10

60. The method according to any one of claims 57 to 59 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 2.

61. The method according to any one of claims 57 to 60 wherein the T-helper epitope comprises an amino acid sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 20.

15

62. The method according to any one of claims 57 to 61 wherein the lipid moiety comprises a lipoamino acid selected from the group consisting of: (i) Pam₁Cys; (ii) Pam₂Cys; (iii) Pam₃Cys; and (iv) Chol₂Lys.

20

63. The method according to claim 62 wherein the lipid moiety comprises the lipoamino acid Pam₂Cys.

25

64. The method according to any one of claims 57 to 63 further comprising producing the lipopeptide.

65. The method according to any one of claims 57 to 64 further comprising determining the immune response of the subject using a sample taken previously from the subject.

30

66. A vaccine against an influenza virus comprising the lipopeptide according to any one of claims 1 to 39 wherein the CTL epitope is from an influenza virus protein.

5 67. Use of the lipopeptide according to any one of claims 1 to 39 in the preparation of a vaccine against an influenza virus.

68. A method of immunizing a subject against hepatitis C virus comprising administering to said subject a lipopeptide comprising a polypeptide conjugated to
10 one or more lipid moieties, wherein:

(i) said polypeptide comprises:

- (a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a CTL epitope of a hepatitis C virus protein, and wherein said amino acid sequences are different;
 - 15 (b) one or more internal lysine residues or internal lysine analog residues for covalent attachment of each of said lipid moieties via an epsilon-amino group of said internal lysine or via a terminal side-chain group of said internal lysine analog; and
 - (c) each of said one or more lipid moieties is covalently attached
20 directly or indirectly to an epsilon-amino group of said one or more internal lysine residues or to a terminal side-chain group of said one or more internal lysine analog residues; and
- (ii) said lipopeptide is administered for a time and under conditions sufficient to elicit a CTL response to said CTL epitope.

25

69. The method of claim 68 wherein the lipopeptide is administered in combination with a pharmaceutically acceptable excipient or diluent.

70. The method of claim 68 or 69 wherein immunological memory is generated
30 against the CTL epitope.

71. The method according to any one of claims 68 to 70 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 176.

72. The method according to any one of claims 68 to 71 wherein the T-helper epitope comprises an amino acid sequence as set forth in SEQ ID NO: 20.

73. The method according to any one of claims 68 to 72 wherein the lipid moiety comprises a lipoamino acid selected from the group consisting of: (i) Pam₁Cys; (ii) Pam₂Cys; (iii) Pam₃Cys; and (iv) Chol₂Lys.

74. The method according to claim 73 wherein the lipid moiety comprises the lipoamino acid Pam₂Cys.

10

75. The method according to any one of claims 68 to 74 further comprising producing the lipopeptide.

15

76. The method according to any one of claims 68 to 75 further comprising determining the immune response of the subject using a sample taken previously from the subject.

20

77. A vaccine against a hepatitis C virus comprising the lipopeptide according to any one of claims 1 to 39 wherein the CTL epitope is from a hepatitis C virus protein.

78. Use of the lipopeptide according to any one of claims 1 to 39 in the preparation of a vaccine against an hepatitis C virus.

25

79. A method of immunizing a subject against *Listeria monocytogenes* comprising administering to said subject a lipopeptide comprising a polypeptide conjugated to one or more lipid moieties, wherein:

30

- (i) said polypeptide comprises:
 - (a) the amino acid sequence of a T helper cell (Th) epitope and the amino acid sequence of a CTL epitope of a *Listeria monocytogenes* protein, and wherein said amino acid sequences are different;
 - (b) one or more internal lysine residues or internal lysine analog residues for covalent attachment of each of said lipid moieties via an

epsilon-amino group of said internal lysine or via a terminal side-chain group of said internal lysine analog; and

- (c) each of said one or more lipid moieties is covalently attached directly or indirectly to an epsilon-amino group of said one or more internal lysine residues or to a terminal side-chain group of said one or more internal lysine analog residues; and
- (ii) said lipopeptide is administered for a time and under conditions sufficient to elicit a CTL response to said CTL epitope.

80. The method of claim 79 wherein the lipopeptide is administered in combination with a pharmaceutically acceptable excipient or diluent.

81. The method of claim 79 or 80 wherein immunological memory is generated against the CTL epitope.

82. The method according to any one of claims 79 to 81 wherein the CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 172.

83. The method according to any one of claims 79 to 82 wherein the T-helper epitope comprises an amino acid sequence as set forth in SEQ ID NO: 20.

84. The method according to any one of claims 79 to 83 wherein the lipid moiety comprises a lipoamino acid selected from the group consisting of: (i) Pam₁Cys; (ii) Pam₂Cys; (iii) Pam₃Cys; and (iv) Chol₂Lys.

85. The method according to claim 84 wherein the lipid moiety comprises the lipoamino acid Pam₂Cys.

86. The method according to any one of claims 79 to 85 further comprising producing the lipopeptide.

87. The method according to any one of claims 79 to 86 further comprising determining the immune response of the subject using a sample taken previously from the subject.

88. A vaccine against *Listeria monocytogenes* comprising the lipopeptide according to any one of claims 1 to 39 wherein the CTL epitope is from a *Listeria monocytogenes* protein.

5

89. Use of the lipopeptide according to any one of claims 1 to 39 in the preparation of a vaccine against *Listeria monocytogenes*.

90. A method of prophylaxis or therapy of cancer comprising administering to
10 said subject a lipopeptide comprising a polypeptide conjugated to one or more lipid moieties, wherein:

(i) said polypeptide comprises:

(a) the amino acid sequence of a T helper cell (Th) epitope and the
amino acid sequence of a tumor-specific CTL epitope, wherein said
15 amino acid sequences are different;

(b) one or more internal lysine residues or internal lysine analog
residues for covalent attachment of each of said lipid moieties via an
epsilon-amino group of said internal lysine or via a terminal side-
chain group of said internal lysine analog; and

20 (c) each of said one or more lipid moieties is covalently attached
directly or indirectly to an epsilon-amino group of said one or more
internal lysine residues or to a terminal side-chain group of said one
or more internal lysine analog residues; and

(ii) said lipopeptide is administered for a time and under conditions sufficient to
25 elicit a CTL response to said CTL epitope.

91. The method of claim 90 wherein the lipopeptide is administered in
combination with a pharmaceutically acceptable excipient or diluent.

30 92. The method of claim 90 or 91 wherein immunological memory is generated
against the CTL epitope.

93. The method according to any one of claims 90 to 92 wherein the tumor-specific CTL epitope comprises the amino acid sequence set forth in SEQ ID NO: 173.
- 5 94. The method according to any one of claims 90 to 93 wherein the T-helper epitope comprises an amino acid sequence as set forth in SEQ ID NO: 20.
95. The method according to any one of claims 90 to 94 wherein the lipid moiety comprises a lipoamino acid selected from the group consisting of: (i) 10 Pam₁Cys; (ii) Pam₂Cys; (iii) Pam₃Cys; and (iv) Chol₂Lys.
96. The method according to claim 95 wherein the lipid moiety comprises the lipoamino acid Pam₂Cys.
- 15 97. The method according to any one of claims 90 to 96 further comprising producing the lipopeptide.
98. The method according to any one of claims 90 to 97 further comprising determining the immune response of the subject using a sample taken previously 20 from the subject.
99. A prophylactic or therapeutic vaccine against cancer comprising the lipopeptide according to any one of claims 1 to 39 wherein the CTL epitope is a tumor-specific CTL epitope. 25
100. Use of the lipopeptide according to any one of claims 1 to 39 in the preparation of a prophylactic or therapeutic vaccine against cancer.